Is adult translocation a credible way to accelerate the recolonization process of *Chondrostoma nasus* in a rehabilitated river?

by

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Abstract. – The decline of the patrimonial rheophilic nase, *Chondrostoma nasus* (Linnaeus, 1758) populations was mainly caused by construction of dams and hydroelectric power-plants, together with the straightening and artificialization of the river banks and water pollution. In this study, we tested the hypothesis whether the translocation of few adult nase individuals from a river stretch to another upstream may be a credible way to accelerate the recolonization process of the species in the Amblève River (Southern Belgium). In February and March 2011, just before their spawning period, eight adult nases (462-509 mm; 1546-2002 g; presumed males and females) were captured in the lower part of the River Amblève. Fin clip samples were stored in alcohol for further genetic analysis. They were equipped with a 14 g radio transmitter and translocated upstream in a 18 km river stretch, where the species had disappeared since decades due to river anthropization. They were manually located two to five times/week using mobile receivers until maximum June 2012 (n = 977 locations). River temperature and flow were hourly recorded during the entire tracking period. The tagged nase individuals displayed various mobility patterns, exploited different areas of the river stretch, occupied longitudinal home ranges from 3.4 to 36.1 km (one individual finally left the new river stretch) and travelled total distances from 12.2 to 186.6 km. The tagged individuals were most of the times apart from one to another, but most individuals grouped together in potential spawning areas in late March-early April 2011, suggesting an attempt to reproduce. In September 2011, electric fishing in two potential detected spawning sites allowed to capture 16 juvenile (0+) nases, demonstrating the existence of spawning activity in the newly occupied river stretch. Individual genetic characterization was performed in 2014 in order to reveal a possible direct lineage between juveniles and adults. Allelic distribution of 22 microsatellite markers unambiguously identified the 16 juveniles as full-sib progeny descending from two of the translocated adults. This demonstrated that the adult nases succeeded to find spawning areas and that progeny found raised-up from the translocated individuals.

Résumé. – La translocation d'individus adultes est-elle une piste crédible pour accélérer le processus de recolonisation de *Chondrostoma* nasus dans une rivière réhabilitée ?

Le déclin des populations de hotus, *Chondrostoma nasus* (Linnaeus, 1758), un cyprinidé rhéophile patrimonial, a principalement été causé par la fragmentation des rivières par la construction de barrages et ouvrages hydroélectriques, ainsi que par l'artificialisation des cours d'eau, les perturbations des débits et la pollution de l'eau. Dans cette étude, est expérimentée la translocation de quelques géniteurs de hotus d'un secteur de rivière à un autre situé plus en amont, afin de déterminer si cette piste présente un intérêt pour accélérer la recolonisation du milieu. En février et mars 2011, juste avant la période de reproduction, huit hotus (462-509 mm ; 1546-2002 g ; présumés mâles et femelles) ont été capturés dans le cours inférieur de l'Amblève (Sud de la Belgique). Des fragments de nageoires ont été prélevés et conservés dans l'alcool pour des analyses génétiques ultérieures. Les individus ont été équipés avec des émetteurs radio d'une masse de 14 g et ont été transloqués en amont, dans un secteur de rivière long de 18 km (entre deux obstacles), où l'espèce avait disparu depuis des dizaines d'années à cause de l'anthropisation du cours d'eau. Les individus marqués ont été localisés de deux à cinq fois par semaine à l'aide d'un récepteur radio mobile, entre février 2011 et juin 2012 (977 localisations). La température et le débit de l'eau ont été enregistrés durant toute la durée du radio-pistage. Les hotus marqués ont montré une grande diversité de mobilité, ont exploité différentes zones du secteur de rivière représentant des domaines vitaux longitudinaux de 3,4 à 36,1 km (un individu a quitté le nouveau secteur de rivière) et ont parcouru des distances cumulées de 12,2 à 186,6 km. Bien que l'espèce soit connue comme grégaire, les individus suivis sont la plupart du temps restés spatialement espacés les uns des autres, mais ils se sont regroupés à certains moments sur des zones de frai potentielles entre mars et début avril 2011. En septembre 2011, des pêches électriques effectuées dans les zones de frai potentielles ont permis la capture de 16 juvéniles de hotus, ce qui prouve l'existence d'un épisode de reproduction dans le nouveau secteur de rivière. En novembre 2014, une caractérisation génétique individuelle a été réalisée pour tenter d'établir un lien de parenté entre ces juvéniles et les adultes transloqués. L'occurrence allélique de 23 marqueurs microsatellites a démontré sans ambiguïté que les 16 juvéniles sont la descendance directe de deux des hotus transloqués. Les résultats démontrent que les adultes transloqués sont parvenus à trouver un site de reproduction et à engendrer une progéniture viable.

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Translocation programs are a common strategy to increase the number of viable populations of threatened freshwater fishes (Vincenzi *et al*., 2012), as insurance against extinction (Minckley, 1995). Recommendations and guidelines for conducting animal translocations have been published with the intention of increasing the success of future translocation efforts (Williams *et al*., 1988; Griffith *et al*., 1989; Dodd and Seigel, 1991; Maitland, 1995). Many fish species are excluded from large parts of their former geographic ranges and neither natural nor unnatural extirpations can be countered by recolonization (Minckley, 1995). Translocations are therefore required to take the place of natural dispersal. On the other hand, dispersal of translocated fish in well-connected systems may be anticipated and desirable to increase range and population size (Minckley 1995). Most translocation attempts have been conducted for a wide array of endangered salmonid fishes (Yamamoto *et al*., 2006) and studies on fish like patrimonial rheophilic cyprinids does not exist despite the important decline of their populations in some rivers due to dam construction and river bed artificialization (Lusk *et al*., 2004; Ovidio and Philippart, 2008).

To date, the evaluation of the success rate of translocation experiments is still low (Harig and Fausch, 2002; DeHaan *et al*., 2011) as it implies quantitative analysis of demographic traits, compensatory responses, life histories and population dynamics of the threatened species (Vincenzi *et al*., 2012). Nevertheless, the understanding of short-term behavioural responses of the translocated fish in their new habitats is a keypoint that may be considered and that will help to understand the feasibility of the method before large scale handling. Strangely, excepted the work of Schemetterling (2003) on cutthroat [*Oncorhynchus clarkii lewisi* (Richardson, 1836)] and bull trout [*Salvelinus confluentus* (Suckley, 1859)], this preliminary behavioural analysis was never really done, and there is an important gap of knowledge on this particular aspect of immediate translocation success mechanisms.

To implement European dispositions, the ecological quality of some priority Belgian rivers improves and fish-passes are constructed from downstream to upstream fragmented stretches. However, the colonization of newly usable habitats is not always obvious for declining species historically adapted to the presence of insurmountable weir. Furthermore, the presence of minimum flow river section downstream some fish-passes are unfavourable and greatly reduce their attractiveness for large rheophilic cyprinids and act as a hydraulic barrier (Ovidio and Philippart, 2008) that still complicates and slows the upstream movements. Using radio-telemetry and individual genetic characterization, we tested the hypothesis whether the translocation of few adult nase, *Chondrostoma nasus* (Linnaeus, 1758), individuals from a river stretch to another upstream, in which the species was present before habitat degradation, may be a credible way to accelerate the recolonization process. Translocated fish were radio-tracked in the new river stretch and particular attention has been given to their behaviour and adaptation in the new environment and their ability to reproduce and raise-up progeny.
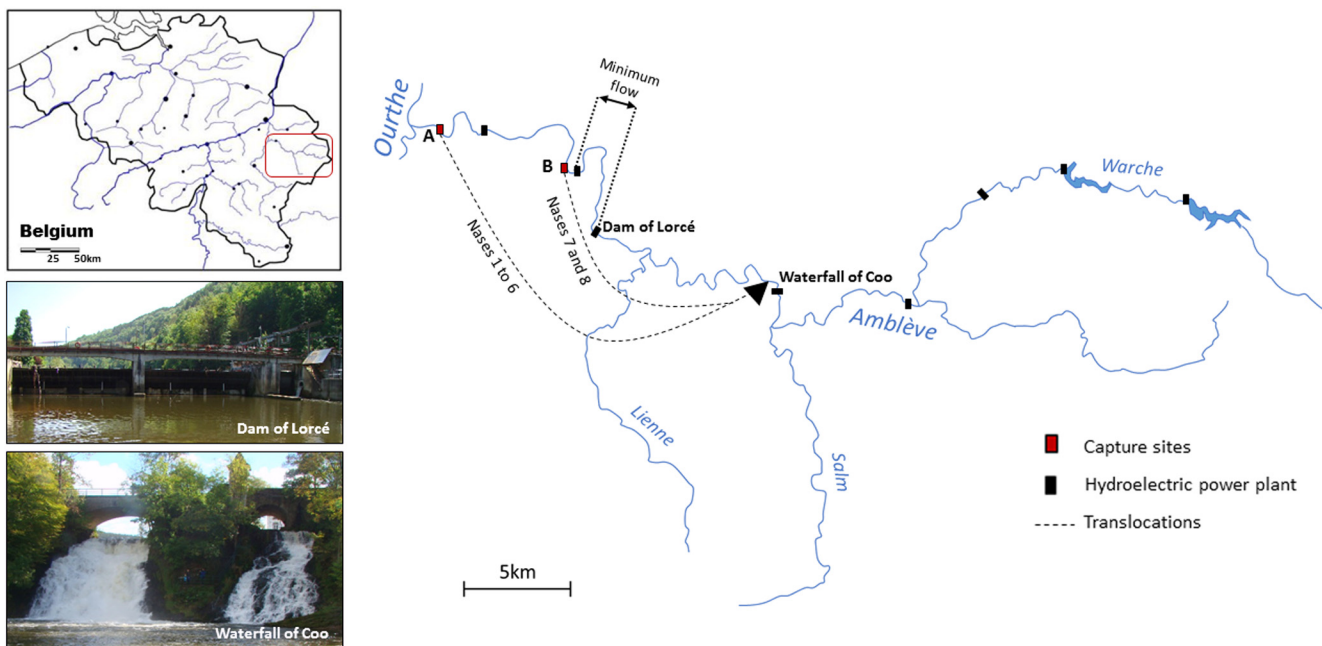

Figure 1. - Map of the study area and representation of the translocations of nase (*Chondrostoma nasus*) performed inside the River Amblève. Letters A and B indicate the two capture sites. The pictures represent the physical barriers in the upstream and downstream limits of the new river stretch.

MATERIAL AND METHODS

Study site, fish tracking and research of progeny

The study was carried out in the lower-middle part of the Amblève River, a tributary of the Ourthe River, Meuse River Basin (Fig. 1). The Amblève is a high quality fast flowing river of 135 km length with a mean annual discharge of 21.7 m³.s⁻¹. The river is fragmented by several major artificial physical barriers and exploited by seven hydroelectric power plants. The study area is located between km 94 and 112 from the source (Fig. 1), which belongs to the grayling zone (Huet, 1949).

Eight nases were captured by boat DC electric fishing in two different parts of the lower Amblève, on 23 February 2011 (n = 6) and 10 March 2011 (n = 2). Nases were anaesthetized in a solution of 2-phenoxy-ethanol (0.2 mg.l⁻¹), and a radio transmitter (ATS Inc., 40 MHz, trailing whip antenna, 14 g) was inserted into the body cavity of the fish through a midventral incision (Ovidio and Philippart, 2008). The ratio transmitter/fish weight was < 1% for each individual (Tab. I). When possible, the sex of the individuals was determined by visual inspection of the gonads through the incision that was closed by three separate stitches, using sterile, resorbable, plain Vicryl sutures. Fin clip samples were stored in alcohol for further genetic analysis. The nases were translocated the day of tagging at the same place, located 24 and 38.5 km upstream from their capture place in a 18 km river stretch, delimited by an 11.8 m insurmountable obstacle in the upstream part, and a hydroelectric power plant on the downstream part (Fig. 1). Nase disappeared from this river stretch since decades due to physical and chemical degradations and habitat fragmentation, but the new fish-pass of the Lorcé dam (Fig. 1) is not used by nase, probably because of a long minimum flow section downstream (Benitez *et al*., 2015). Fish tracking started the day after tagging. Locations were determined by triangulation from markers on the banks of the river, using mobile FieldMaster radio receivers and loop antennas (ATS Inc.) from February 2011 to June 2012. Locations (n = 977) were made during daytime, with

accuracy between 5 and 20 m², depending on the distance between the fish and the observer and the width of the river. Fish were located from two to five days a week, with more intensity during the *circum* reproduction period. Water temperature was recorded hourly by data loggers (TidBit; Onset Computer Corp.) and water flow was continually monitored (data from the Water Division-SPW).

On 21 September 2011, an electric fishing near a potential detected spawning area (6.4 km from the release site) was performed in order to capture progeny potentially derived from the translocated adults.

Parentage testing

Total genomic DNA was extracted from fin clip sampled on translocated adults and juveniles fished in the studied stretch (see results below) using DNeasy Blood & Tissue kit (Qiagen). Genetic characterization was based on 22 microsatellite markers: *BL1-2b, BL1-30, BL1-84, BL1-153, LleA-029, LleA-071, LleC-090, LceC1, Lsou19, LleA-150, Lsou05, Lsou08, Lsou29, Lsou34, Ppro132, CnaB-030, CnaD-112, CtoA-247, CtoA-256, CtoE-249, LCO3, Rser10*. Primer sequences and multiplex PCR sets were described in Dubut *et al*. (2010). PCRs were carried out in 10 μl volumes containing between 0.05 and 0.3 μL of each 10 μM diluted primer (5'-end fluorescently labelled forward and unlabelled reverse), 5 μl Multiplex PCR Master Mix (Qiagen) and 1 μl DNA. Amplification conditions were: 95°C for 15 min, followed by 30 cycles (denaturation at 94°C for 45 s, annealing at 57°C for 90 s, extension at 72°C for 1 min) and a final extension step at 72°C for 30 min. PCR products were genotyped on a ABI 3130XL Genetic Analyzer using GeneScan-500 (LIZ) size standard and microsatellite length variation was scored using GeneMapper 4.0 software (Applied Biosystems).

Sibling relationship among 0⁺ juveniles and parentage assignment with translocated adults were assessed using COLONY 2.0 software (Jones and Wang, 2009). COLONY is designed to infer sibship and parentage among individuals using their multilocus genotypes and a maximum likelihood

Table I. - Characteristics of the individual nases (*Chondrostoma nasus*) tracked in the Amblève River and values of their mobility indicators.

Individuals	FL (mm)	Weight (g)	Sex	Tag/body weight ratio	Date capture (site)	End of tracking	Localizations (n)	Longitudinal home-ranged (km)	Distance travelled (km)
Nase 1	490	2002	F	0.69	23/02/2011(A)	20/02/2012	119	9.85	17.0
Nase 2	465	1786	M	0.78	23/02/2011(A)	01/03/2012	144	16.97	186.7
Nase 3	462	1561	F	0.89	23/02/2011(A)	28/06/2012	158	3.44	15.74
Nase 4	475	1625	F	0.86	23/02/2011(A)	05/09/2011	82	16.94	19.56
Nase 5	484	1625	F	0.86	23/02/2011(A)	28/06/2012	160	3.47	15.63
Nase 6	509	1885	F	0.74	23/02/2011(A)	14/07/2012	67	8.25	12.28
Nase 7	456	1546	M	0.90	10/03/2011(B)	19/03/2012	116	9.17	38.60
Nase 8	476	1621	M	0.86	10/03/2011(B)	22/05/2012	131	36.19	97.03

method. Parameters were set as follows: mating system: male and female polygamy, inbreeding present; analysis method: full-likelihood; no sibship prior; no known and no excluded sibship; other parameters set as default.

RESULTS

Fish behaviour

From the beginning of the study (23 February 2011) to late November 2011, the flow regime of the Amblève River was atypically plane and low, continuously < 6 m³.s⁻¹ (Fig. 2). From late November 2011 to the end of the study in June 2012, the flow level was more fluctuating with major

peaks from mid-December 2011 to February 2012. In spring 2012, the flow was higher and more unstable than in spring 2011. In 2011, the water temperature reached for the first time the level of 10°C in late March, while in 2012 it happened only in late April (Fig. 2).

Just after the translocation in late February 2011 until mid-March, the nases remained grouped near their release place, just downstream the Coo barrier (Fig. 2). Once the water temperature has reached 10°C in late March, most individuals first tried to move upstream but were blocked by the waterfall and finally moved in downstream direction. On some places (3.6 km, 6.4 km and 17.1 km from the release site) all nases, except no 7, were observed by couple or group in favourable spawning habitats between 17 March
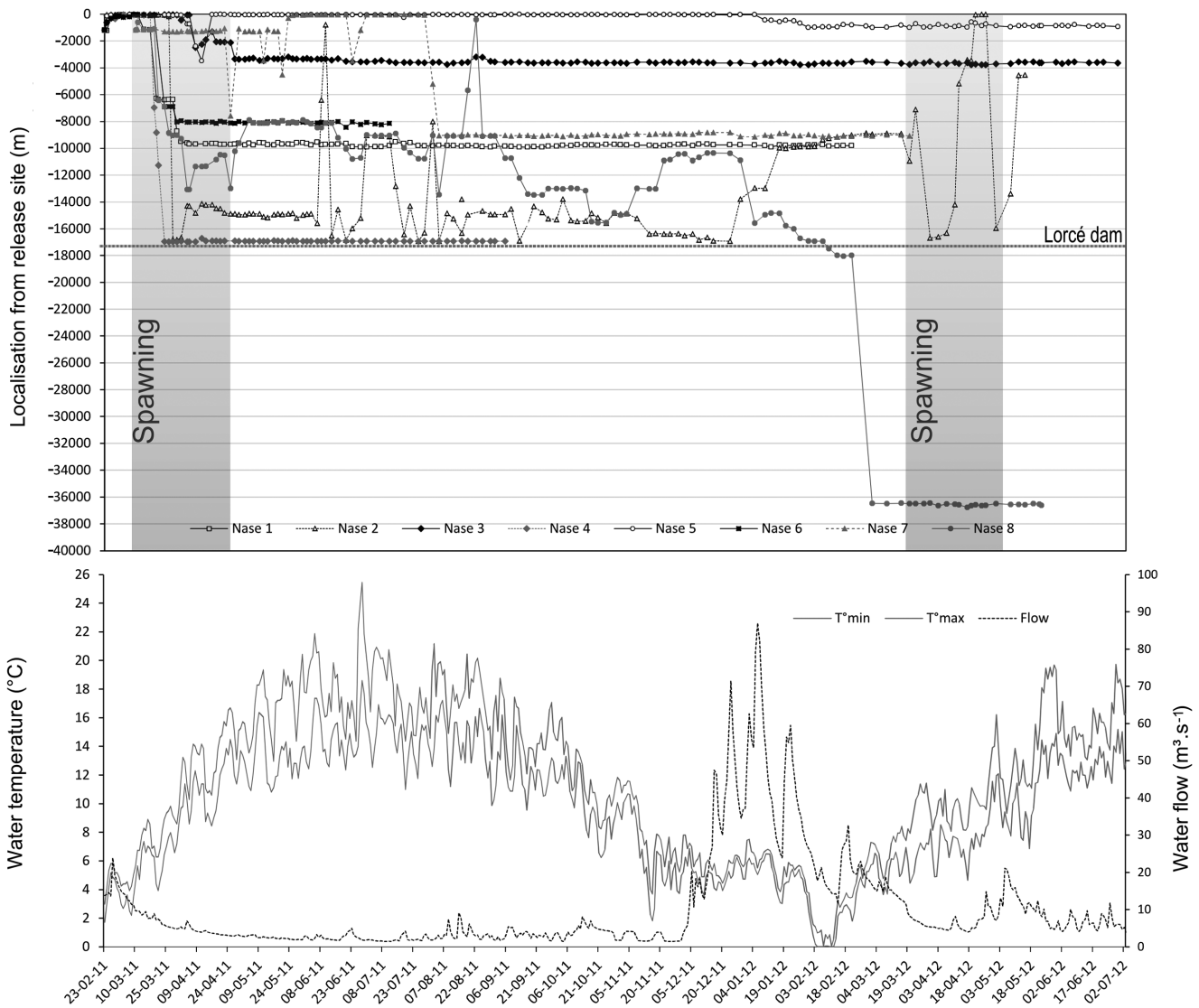

Figure 2. - Movement behaviours of eight translocated nases in the River Amblève between February 2011 and July 2012 in relation with the variations in water temperature and flow. In the upper graph, point 0 corresponds to the release site and dark rectangles represent the identified spawning period.

and 11 April 2011. After the 2011 spawning period, they remained dispersed all over the 18 km stretch of the River and stayed most of the time apart from one to another. Individuals no 1, 3, 4, 5, 6 and 7 were less mobile and remained within the same morphodynamic unit of the river. Nase no 2 and 8 showed frequent long range up and downstream movements and nase no 8 finally left the river stretch during an important spate. During the 2012 spawning period, nase no 2 joined nases no 3 and 5 in a potential spawning site 0.9 km and 3.6 km from their initial release site. When considering the entire tracking period, longitudinal home ranges varied from 3.4 to 36.1 km and total longitudinal distance travelled from 12.2 to 186.6 km (Tab. I).

Research of progeny and parentage testing

A total of 16 juveniles were captured (fork length: 53.9 ± 3.6 mm; weight: 1.51 ± 0.36 g) by electric fishing (Fig. 3). They were identified as full siblings with a probability of 1.0. The number of different alleles at each locus was comprised between 2 and 4. Two of the translocated adults (individuals no 2 and 6) were assigned as parents of these offspring with a probability of 1.0.

DISCUSSION

After their transportation in a unique site in the new river stretch, just some days before their potential reproduction period – late March to mid-May in the Amblève (Ovidio and Philippart, 2008) –, we demonstrated that the adult nases succeeded to find spawning areas and that progeny found raised-up from the translocated individuals. In its natural home range, nase is a species that mainly migrates in the upstream direction to spawn and performs later post-spawning downstream movements (Hubert and Kirchhofer, 1998; Ovidio and Philippart, 2008). In this study, as individuals were transferred close to their reproduction period, and because migration in the upstream direction was not possible due to the presence of an insurmountable weir in the upper part of the new river stretch where they have been transferred, the nases moved atypically in the downstream direction to spawn. Although previous studies on the rheophilic cyprinid barbel [*Barbus barbus* (Linnaeus, 1758)] showed a strong fidelity to spawning sites within their natural home range (Ovidio *et al.*, 2007), we demonstrated that translocated nases were able to find alternative spawning areas in the new river stretch. Nases were also able to meet to spawn at a density of eight individuals for 18 km of river stretch, despite the fact that they were split one from each other most of the time during the tracking period. This grouping behaviour maybe involves an unknown remote recognition mechanism that allows spread individuals to join for spawning. It was unfortunately impossible to visually determine if the tracked individuals joined shoal of other large rheophilic species [e.g. barbel, and chub, *Squalius cephalus* (Linnaeus, 1758)] to form heterospecific shoal in some instances. However, the three species do not spawn at the same moment (Ovidio and Philippart, 2002) and a positive influence on nase reproduction is not evident. As translocated nases belonged to a homogenous genetic lineage spread in the Belgian Meuse basin (Gennotte *et al.*, 2014), a certain degree of genetic relatedness may also facilitate the individual recognition, grouping and spawning synchronization. Anyway, the reproduction success of the translocated individuals was probably favoured by the fact that we kept them in the same river, with similar water and habitats quality as in their capture place, but it emphasizes a kind of short-term behavioural plasticity never documented in nase.

More broadly, the success of reintroduction programs is commonly assessed in long-term monitoring of survival, spawning and recruitment (Cochran-Biederman *et al.*, 2015). In this study, the use of genetic kinship analysis brought rapid evidence of the reproduction of translocated adults and their progeny. The recovered juveniles only originated from two individuals, but all potential spawning grounds and refuge areas have not been prospected by electric fishing, and other individuals have been able to reproduce. Even if different studies were performed on genetic diversity of fish population after translocations events (Stockwell and Leberg, 2002; Eldridge and Naish, 2007) the discovery of direct progeny of translocated mature adults has never been considered yet.

As a test study, the number of translocated individuals is probably not sufficient to engender sustainable populations and to ensure long-term maintenance of genetic variability (Cross, 2000) but further translocations may follow in the future. However, the minimum number of individuals to support a self-sustaining population is specific to particular species or populations (Yamamoto *et al.*, 2006) and objective rule does not really exist. Yamamoto *et al.* (2006) observed that the translocation of 20 individuals white-spotted charr [*Salvelinus leucomaenis* (Pallas, 1814)] was sufficient to restore genetic diversity above a dam in the Kame and Hitozuminai rivers (Japan). Some recommendations and guidelines for conducting animal translocations have been published with the intention of increasing the success of future translocation efforts (Williams *et al.*, 1988; Griffith *et al.*, 1989; Dodd and Seigel, 1991; Maitland, 1995). The number of individuals released must be adequate (but not objectively defined), should preferably consist of several age classes of wild-caught individuals, and should be released in areas with suitable habitat. Other factors to be considered are sex ratio of natural populations, reproductive season and fecundity, and presence of congeneric species or other potential competitors at introductions sites (Joly, 2003).

Figure 3. - **A**: Habitat where young of the year nases were captured in September 2011 in the River Amblève. **B**: Juvenile nase captured in September 2011.

As a first approach on rheophilic cyprinids, this preliminary test reveals that intra river translocation of adult individuals potentially operates and may be a credible way to accelerate a colonization process, if good quality spawning habitats are available. Translocation is simpler to implement than artificial hatching, that requires important infrastructures and human resources, and may be an alternative when downstream populations are still abundant. However, as translocations may have adverse consequences if basic rules are not followed (Maitland and Lyle, 1992; Maitland, 1995), this technique cannot be considered without a well thought long-term restoration program at the scale of the entire river basin, a scientific monitoring of the restored populations and serious knowledge on the biology of the target species.

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